

# Charting a Course for our Professions (Environment):

## ARMs Associated with Fecal Wastes from Food Animal Production and Human Sources

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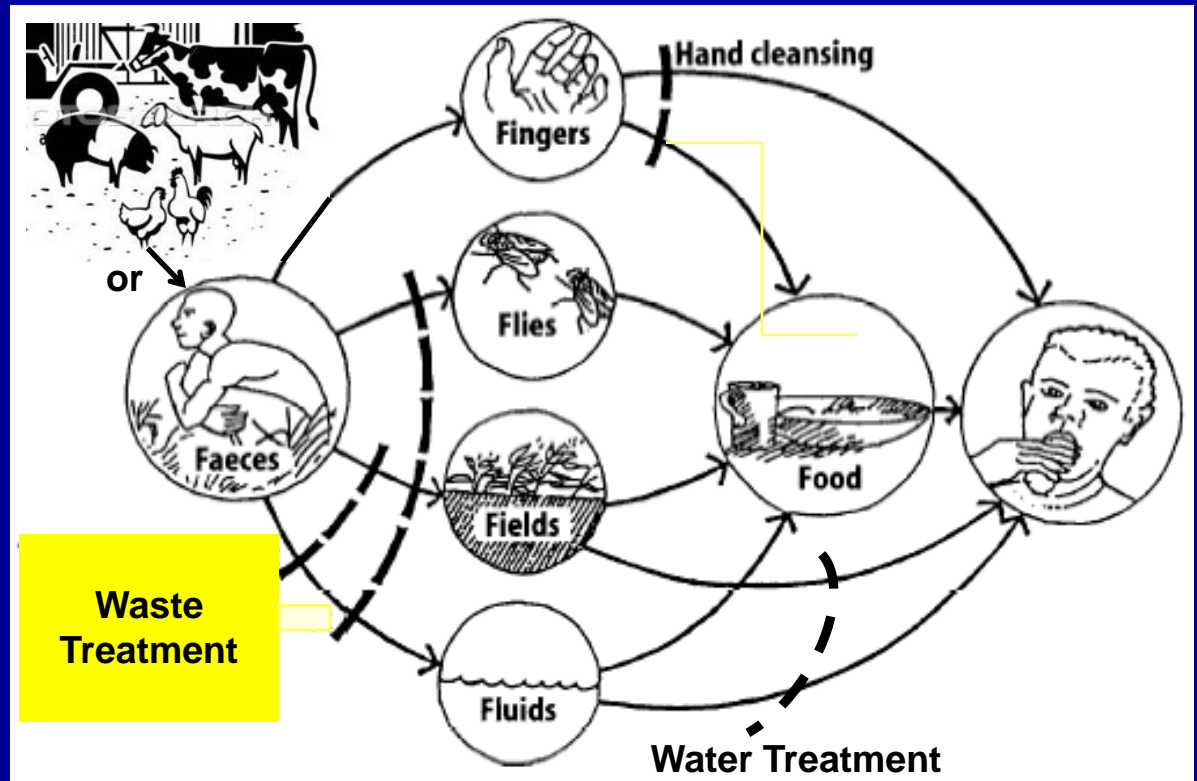
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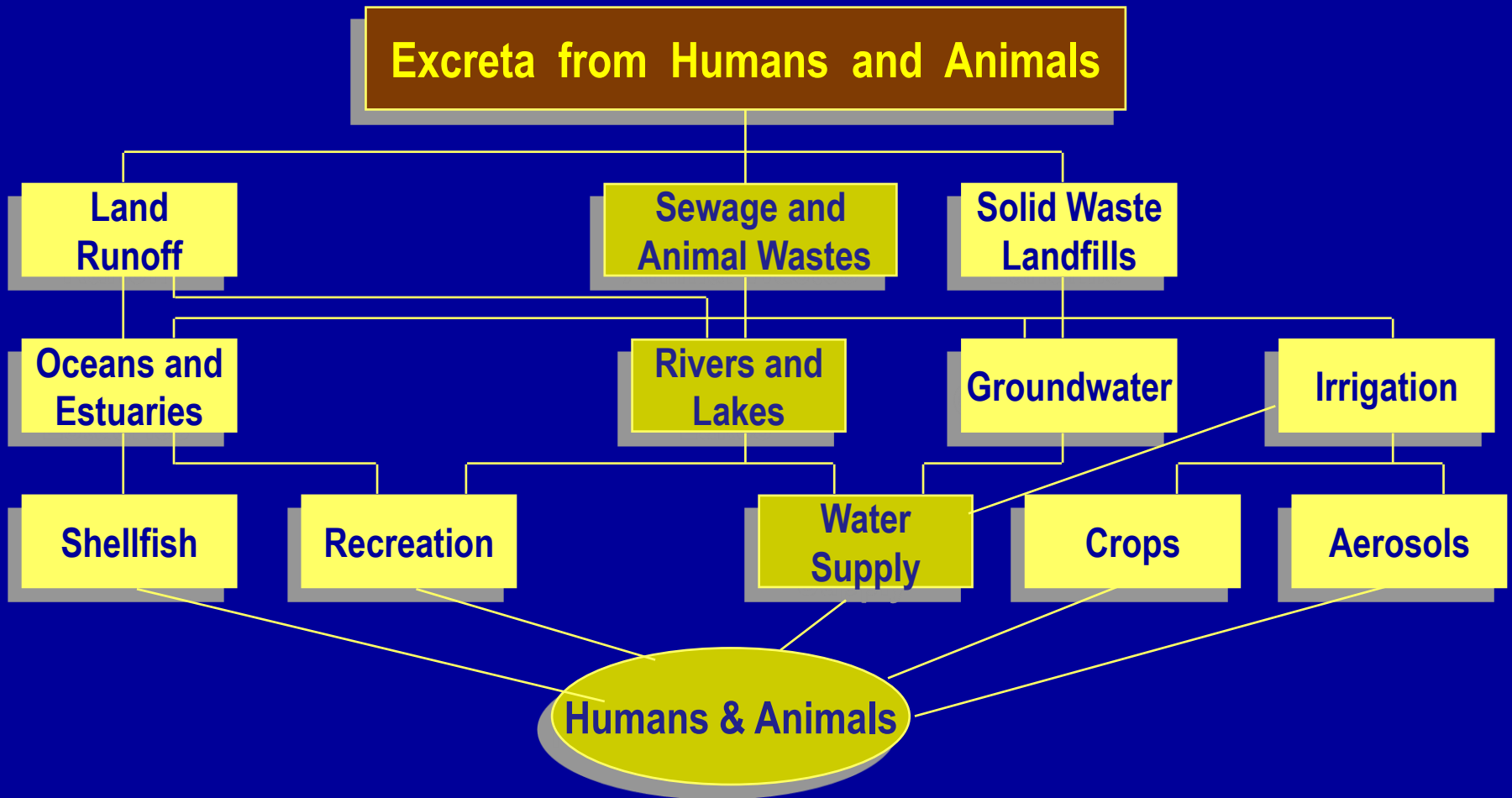
# Why We Must Care about Health Risks from Antimicrobial Resistant Microbes from Human and Animal Sources

## The F's of WaSH and Interventions for them:

- Feces
- Fingers
- Flies
- Fields/Food
- Fluids
- Fomites



# Enteric Pathogens Can Come from Human and Animal Feces by Various Routes of Exposure



# Human and Animal Wastes Contain High Concentrations of Antimicrobially Resistant Enteric Bacteria (Pathogens)

- Colonization and fecal shedding of high levels of AR bacteria that are potentially pathogenic for humans
- Pathogen levels in human and animal wastes can be millions to billions per gram of feces
- Zoonotic: many agricultural animal bacteria/pathogens can infect and cause disease/death in humans: zoonotic
  - Foodborne transmission is well-documented



# Antibiotic Resistance of *E. coli* on NC Swine Farms

High percentage of isolates from test farms are resistant to CTET and TET

Most isolates from conventional (surrogate) farms and alternative technology farms were resistant to multiple antibiotics

Antibiotic	Surrogate Farm 1 (n=19)	Surrogate Farm 2 (n=31)	Barham Farm (n=23)	Super Soils (n=17)
STR	21%	19%	43%	29%
CTET	100%	100%	96%	100%
TET	100%	100%	96%	100%
TMP	0%	0%	9%	0%
SMX	32%	29%	35%	12%
CHL	21%	29%	9%	24%
CIP	0%	0%	4%	0%
GEN	0%	0%	0%	0%
AMP	32%	16%	13%	18%

Farm	% of total isolates with MAR	Total # of isolates
Surrogate Farm 1	100%	19
Surrogate Farm 2	100%	31
Barham Farm	91%	23
Super Soils	100%	17

# Antibiotic Resistance of *Salmonella* on NC Swine Farms

High percentage of isolates from studied farms are resistant to CTET and TET

Most isolates from conventional (surrogate) farms and alternative technology farms were resistant to multiple antibiotics

Antibiotic	Surrogate Farm 1 (n=19)	Surrogate Farm 2 (n=31)	Barham Farm (n=23)	Super Soils (n=17)
STR	45%	55%	7%	50%
CTET	90%	94%	60%	100%
TET	85%	94%	57%	100%
TMP	0%	3%	23%	0%
SMX	35%	88%	37%	45%
CHL	30%	18%	3%	25%
CIP	0%	0%	0%	0%
GEN	0%	0%	0%	0%
AMP	25%	21%	10%	30%

Farm	% of total isolates with MAR	Total # of isolates
Surrogate Farm 1	85%	20
Surrogate Farm 2	100%	33
Barham Farm	57%	30
Super Soils	100%	20

# Factors Influencing Antimicrobial Pathogen Risks from Food Animal Production & Human Waste Systems

- Increasing/continued use of antibiotics in animals and humans
- Continued dietary antibiotic use for animal growth promotion
- Greater concentrated animal husbandry practices (CAFOs)
- Anthropogenic changes in food animals/their zoonotic pathogens
  - Animal breeding stock
  - Human medical therapeutic use
  - *E. coli*, enterococci, campylobacters; salmonella, MRSA, etc.
- Population factors
  - High animal numbers and density, overcrowding, colonization of gut flora, transfer among animals and to people; asymptomatic carriage
- Environmental factors
  - *Environmental releases (wastes, water, land, air), microbe and antibiotic persistence, AR mutation/selection, AR gene transfer among pathogens/other bacteria, human and animal waste management*
- Impacts of climate and weather events; ↑frequency and severity

# **Animal Wastes are Major Reservoirs/Sources of Antimicrobially Resistant Bacteria**

**Daily “Manure” Production by Many Food Animals Exceeds That of Humans**

<b><u>Animal</u></b>	<b><u>Grams Wet Weight</u></b>	<b><u>% Moisture</u></b>
<b>Human</b>	<b>1,500</b>	<b>77</b>
<b>Cow</b>	<b>30,000</b>	<b>85</b>
<b>Hog</b>	<b>4,000</b>	<b>71</b>
<b>Sheep</b>	<b>1,500</b>	<b>75</b>
<b>Turkey</b>	<b>430</b>	<b>68</b>
<b>Chicken</b>	<b>140</b>	<b>73</b>

# Fecal Waste Production of Swine and Humans: e.g., North Carolina Waste Production & Loads

- Per capita, swine produce about **5 times** as much feces as humans (more-or-less)
- NC's swine population is 9-10 million animals
- NC's human population is 9-10 million
- So, feces production in NC is much higher from swine than humans; other ag. animals adds even more feces/AR microbes
- **Fecal wastes of humans and agricultural animals are managed very differently**
  - Extensive human sewage Rx/disinfection and microbial limits
  - Minimal animal waste Rx and no microbial requirements or limits
    - **“non-discharge” systems(?)**

# Animal Wastes that Contain AR Pathogens and General Management Approaches

- Waste types: feces, urine, carcasses, birthing wastes, and airborne particles (droplets and bioaerosols), etc.
- Solids management: utilize/dispose by land application; dewater, store/cure, **compost (rare), land apply, market**
- Liquids management: treat/store (currently in lagoons); then utilize/dispose (mostly by land application)
- Carcass Management: bury, render, **compost, incinerate**
- Airborne particles management: droplets and aerosols discharged to atmosphere; controlled **sedimentation or impact collection is rarely used**

# Animal Manure Management Systems and Environmental Impacts by AR Bacteria

- Water, soil and air pollution are documented impacts
- Typical animal production and manure management systems have resulted in environmental pollution
- Typical liquid waste management:
  - Store in anaerobic lagoons (seepage to groundwater)
    - Months are required (but sometimes only weeks or days occur)
  - Then land applied (sprayfields, drains, injection wells, ditches)
  - Feedlots, pastures, loafing lots, etc.

- **On and off-farm AR microbial fecal contamination occurs**
- **Impacts on drinking, bathing, fishing and irrigation waters**
  - **Especially when there is precipitation (rainfall/storms)**

# Some Other Documented Pathways for ARM Pathogen Contamination from Food Animal Farms

## Airborne transport: bioaerosols

- from hog house exhaust
- during land application of waste by spray irrigation
- Enteric microbes isolated from aerosols up to 1000s of feet from spray irrigation equipment
- Downwind microbial concentrations exceed upwind microbial concentrations
- Deposition on land/water

## Mortalities: burial of dead animals

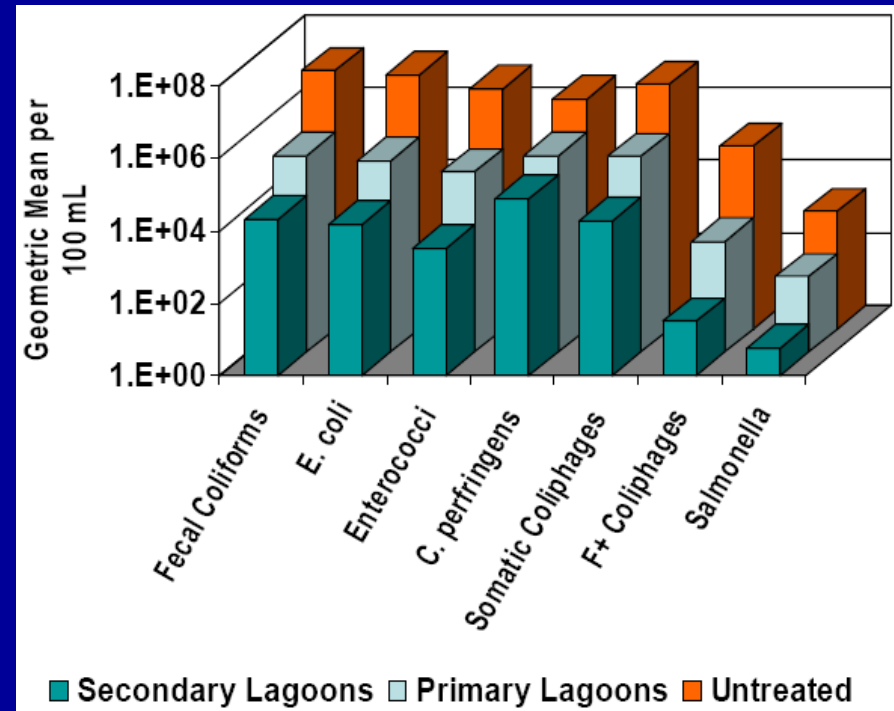
## Produce: by irrigation with waste

# Treatment Practices/Management and Microbial Quality of Human and Ag. Animal Fecal Wastes Differs

- Animals:
  - Storage and liquid or solid waste land application
  - No requirements for or limits on microbial quality
- Humans:
  - Multi-step treatment requirements (of EPA/states)
    - Physical, biological & chemical treatment processes
  - Microbial quality requirements for both water discharge and land application
    - Wastewater effluent microbial quality requirements
    - Biosolids microbial quality requirements

# Microbial Reductions by Anaerobic Lagoon Treatment of Swine Waste: Moderate, Not Great

- Lagoons maintained/operated by BMPs moderately (~90-99%) reduce fecal bacteria and viruses
- Fecal indicators in lagoon effluent: 1000s to 100,000s or more/100 ml
- Exceed state standards and federal guidelines for fecal coliform levels in municipal wastewater effluent or sludge (biosolids) applied to land or sewage effluent discharged to water
- *Salmonella* in lagoon effluents: 10s to 100s to 1000s or more per 100 ml.
- *Yersinia enterocolitica* and hepatitis E virus detectable in 100-ml or 1-liter volumes of lagoon liquid



Effects of BMP Treatment by Anaerobic Lagoons on Enteric Microbes Levels in Lagoon Liquid

**We Could Do Far Better!**

# Swine Waste Management and Pathogens: Better Lagoon Treatment is Possible

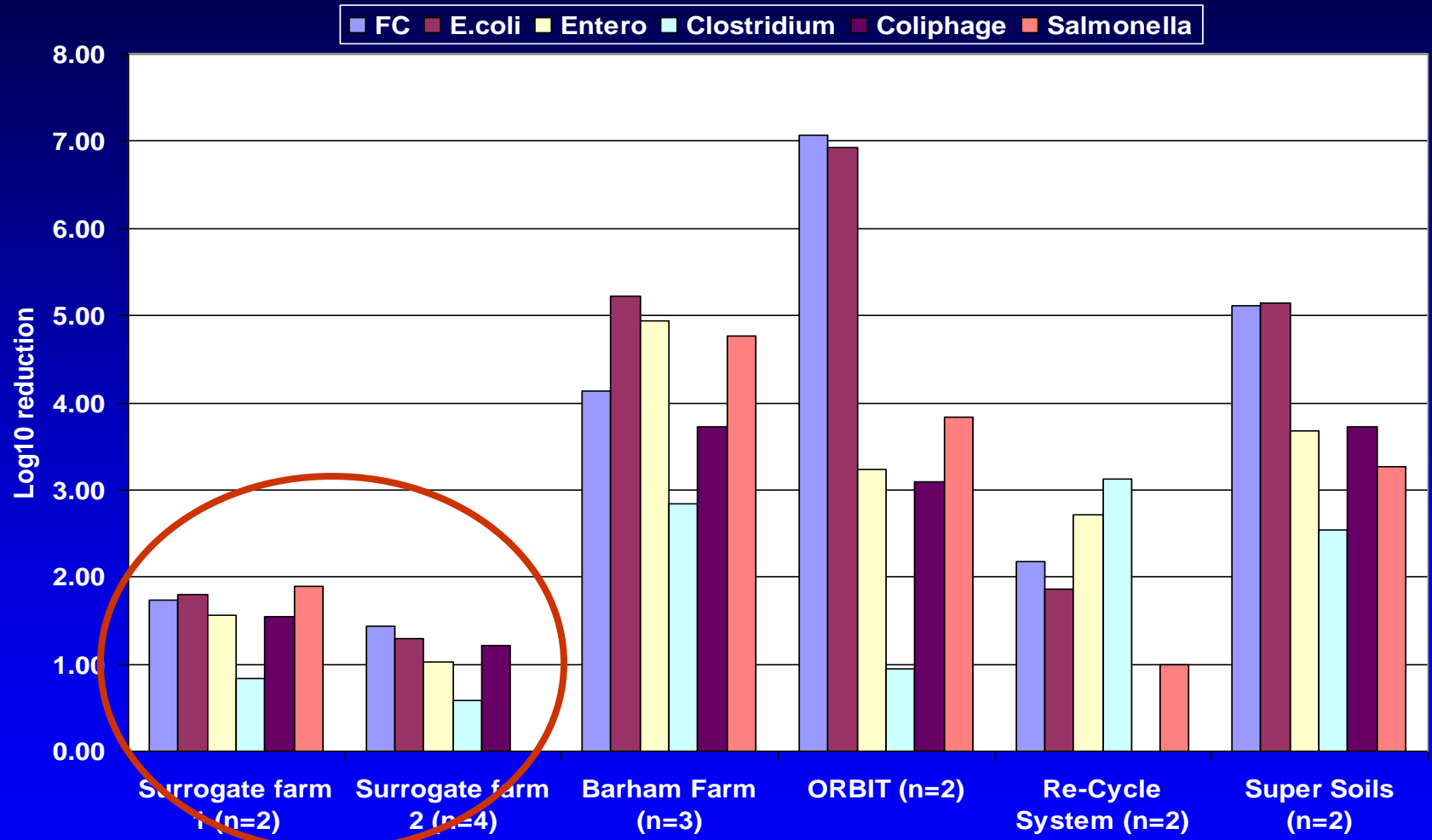
- **Two-stage lagoon systems:**
  - **Enteric bacteria and virus reductions of 99-99.9%, including *Salmonella***
- **Additional pathogen reductions achieved with additional lagoons in series**
  - **Even greater microbial reductions can be achieved by 90-99% per additional lagoon**

# Animal Liquid Waste Treatment/Management and Microbes: Better Alternatives to Lagoon Rx

## Anaerobic digestion:

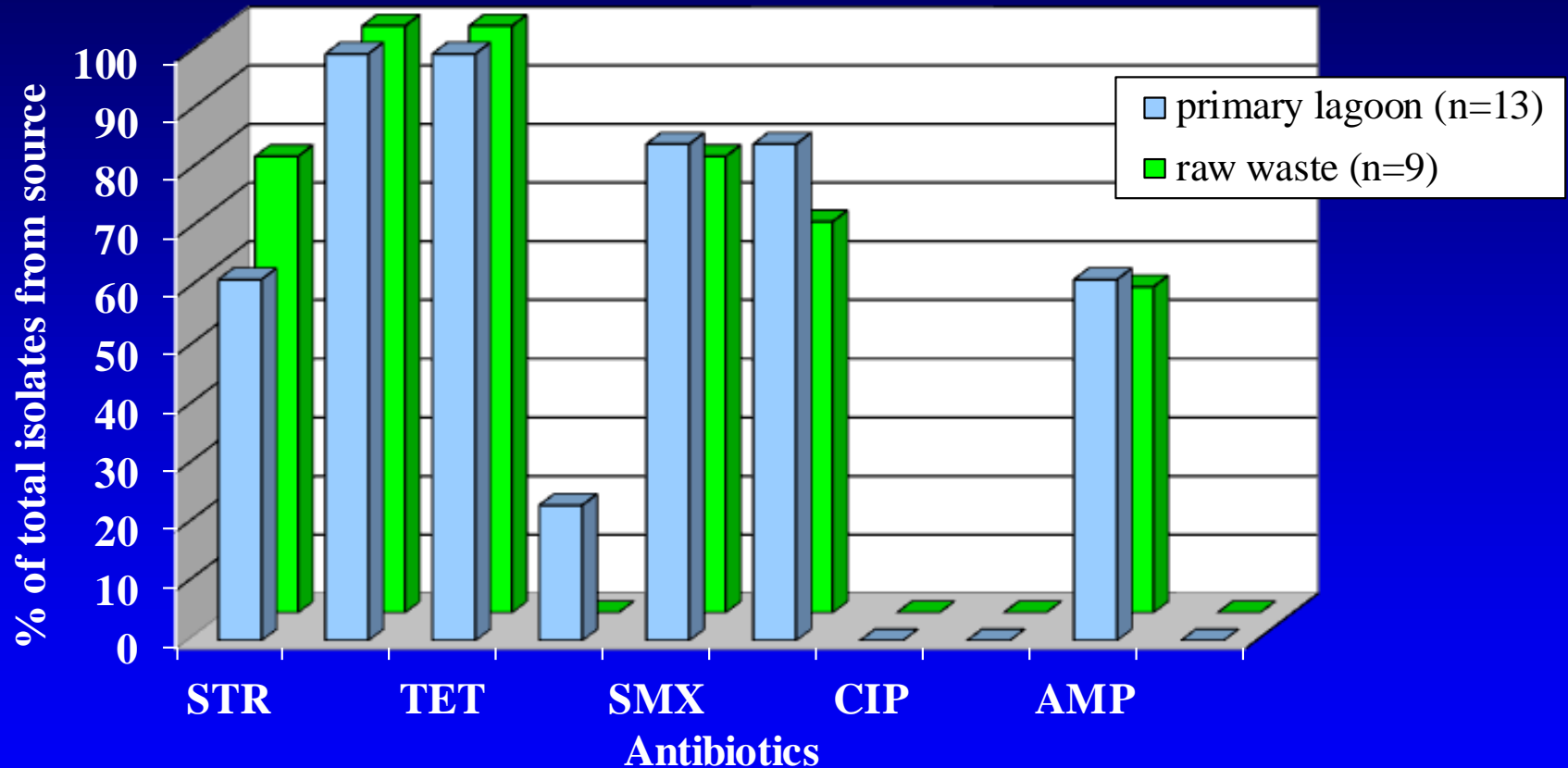
- Thermophilic:
  - 3-6  $\log_{10}$  (99.99-99.9999%) reduction of fecal bacteria
  - $>3 \log_{10}$  reduction of viruses
  - $>3 \log_{10}$  reduction of *Cryptosporidium parvum*
- UV irradiation: 99-99.9% reduction of enteric bacteria and viruses in biologically treated liquid waste
- Inadequate studies on full-scale systems

# Microbial Reductions in Total Waste Residuals from Swine Waste Treatment by Conventional and Alternative Technologies: Alternative Technologies Provide Greater Reductions than Current Technology



Alternative systems (Barham, ORBIT, Re-Cycle and Super Soils) show considerable promise for reducing microbial pathogens in swine waste when compared to the surrogate systems

# AR *Salmonella* are Present in Both Raw and Treated Swine Waste



**Little Change in AR Frequency by Conventional Swine Waste Rx**

# **Animal Solid Waste Rx/Management and Pathogens: Alternative Technologies will Better Reduce AR Microbes**

- Enteric microbe levels decline in stored manure
  - ...but only slowly and generally not extensively
- Dry storage gives some pathogen reduction
  - But, reductions are poorly characterized for different conditions and pathogens
- Composting increases pathogen reduction
  - >99.99% has been achieved
- Land application method and rate influences enteric microbe survival, transport and fate in soil
  - survival, transport and fate is poorly characterized
- Stored manure can attract vectors
  - Pathogen recontamination by animals and other vectors (e.g., birds, mice, flies) is a concern
  - Vectors can also spread pathogens

# **Microbial Reductions by Animal Waste Treatment Processes and Management Systems: Do Exposures Result in Pathogen Risks to Humans?**

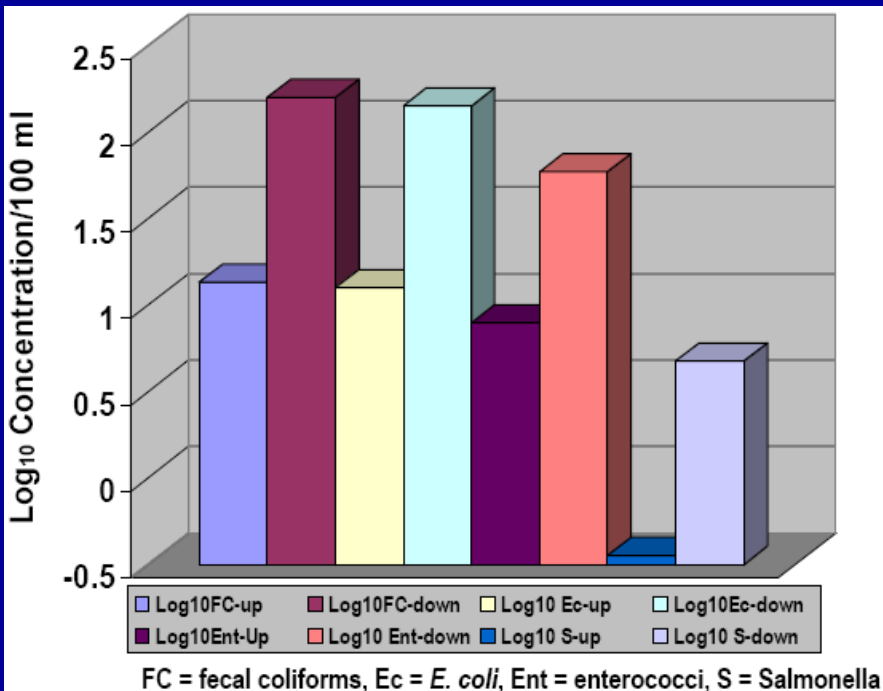
- **Most ag. waste Rx studies have been only for fecal indicator bacteria**
  - **Little data on bacterial pathogens, viruses & parasites**
- **Most rxs. achieve only modest (90-99%) AR microbial reductions**
  - **High microbe levels remain in treated liquids and solids**
- **Many BMP systems are ineffective for AR microbial reductions**
- **Survival, transport and fate of microbes after land application and other post-treatment management approaches is poorly characterized**
- **Environmental contamination occurs and has been documented; human waterborne outbreaks have occurred**

## **Q: Are there Microbial Impacts of AFOs and CAFOs on Environmental Quality?**

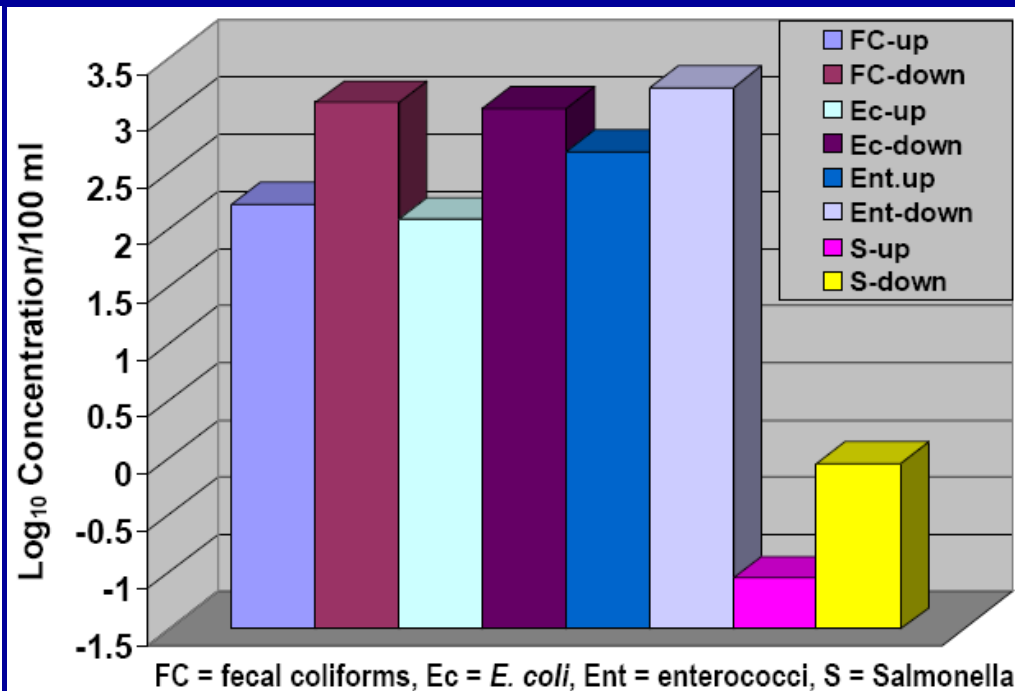
**A: Animal agriculture often increases enteric microbe levels in surface and ground waters:**

- Direct waste discharges to surface waters (illegal in some states; but violations occur)**
- Drainage, seepage or spills from lagoons or other treatment/storage systems**
- Drainage or runoff from land-applied liquid and solid wastes**
- Increased enteric microbe levels in subsurface groundwaters nearby surface waters**
  - Further increased 10-100X by storm events**
  - Result in further exceedance of state and Federal microbial standards for recreational and drinking source water quality**

# Impact of Food Animal Production Systems on Fecal Bacteria Levels in Surface Waters: Up- and Down-stream Bacteria Levels



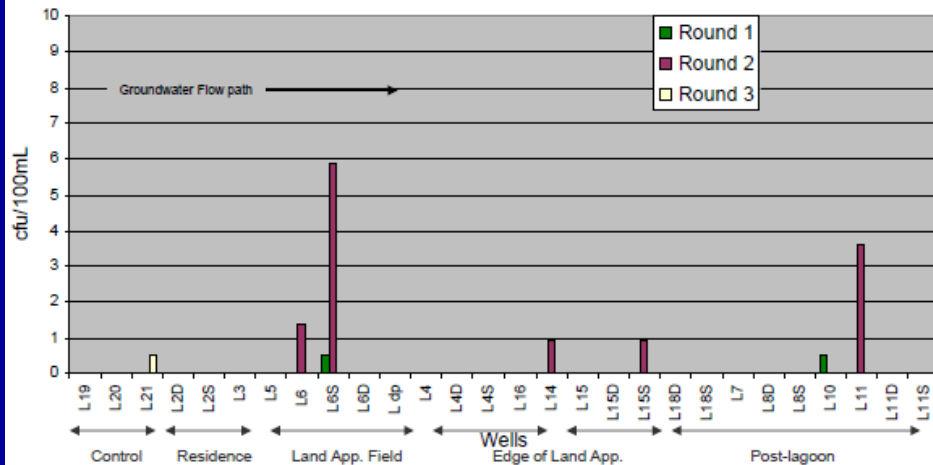
**Nursery Farm  
Duplin County, NC**



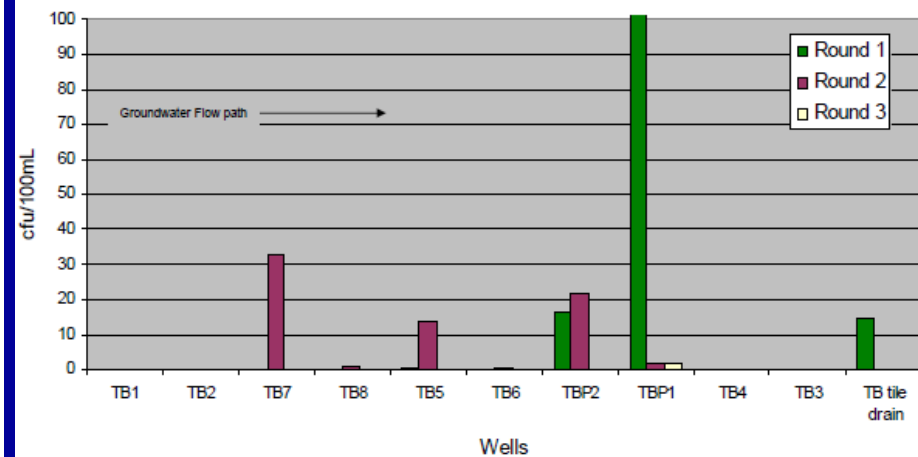
**NCSU Demonstration Farm  
Raleigh, Wake County, NC**

# *E. Coli* and Enterococci are Present in Groundwater of NC Swine Farms - More than in Non-animal Control Farms

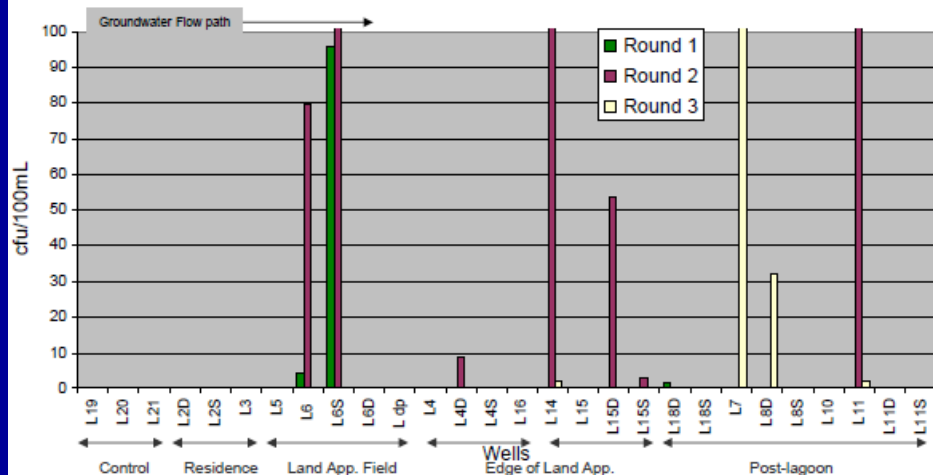
*E. coli* Concentrations - Swine Farm #1



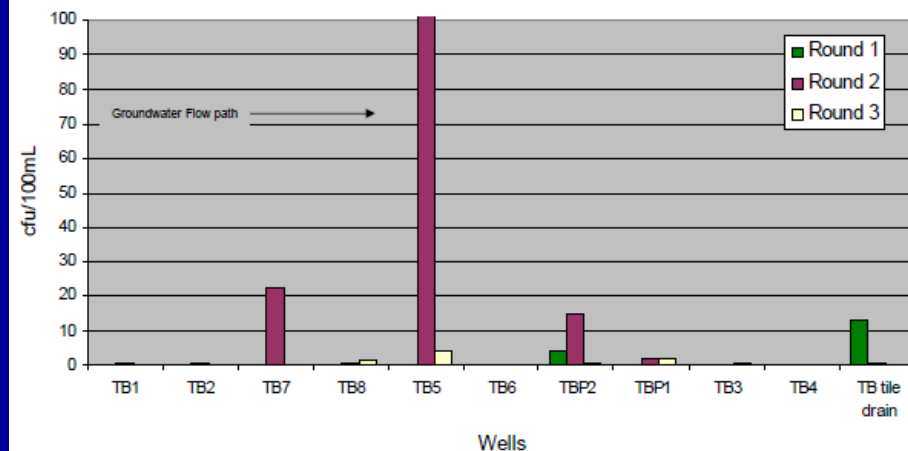
*E. coli* Concentrations - Swine Farm #2



Enterococcus Concentrations - Swine Farm #1



Enterococcus Concentrations - Swine Farm #2



# Significant Bacterial Impacts on Groundwater Quality by Swine Farms vs. Reference Farm

## Kruskall-Wallis $\chi^2$ Test – *E. coli*

- For statistical analyses, significant differences are:
  - $p = 0.01$  is strongly significant
  - $p = 0.05$  is significant
  - $p = 0.05$  to  $0.1$  is moderately significant
  - $p > 0.1$  is not significant.
- Statistically significant differences in bacterial concentrations between:
  - Swine #2 v. Reference (moderately sig.).

	Swine #1	Swine #2	Pooled	Land App.
Reference	0.50	0.07	0.25	0.94
Pooled Sw				0.19

## Kruskall-Wallis $\chi^2$ Test – Enterococci

- Statistically significant differences in bacterial concentrations between:
  - Land application v. Reference site (strongly sig.).
  - Pooled Swine farms v. Land application site (strongly sig.).

	Swine #1	Swine #2	Pooled	Land App.
Reference	0.41	0.58	0.35	0.007
Pooled Sw				0.01

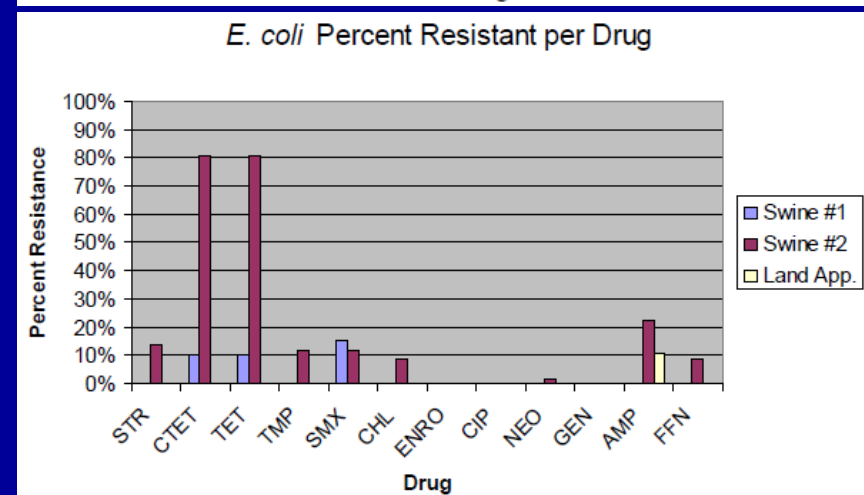
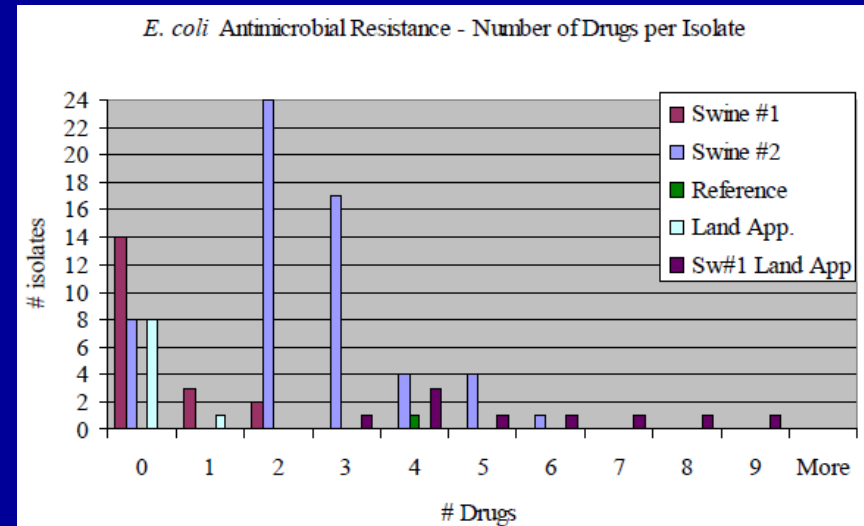
# AR Frequencies and MAR of *E. coli* Bacteria from Swine and Reference Sites

Significant impacts of AR occurrence and MAR in *E. coli* on the groundwater quality of swine farms versus reference farm

## Wilcoxon Scores Exact Test – *E. coli*

- Statistically significant differences in AR frequency between:
  - Swine #1 v. Reference site (moderately sig.)
  - Swine #2 v. Reference site (strongly sig.)
  - Pooled Swine farms v. Reference site (strongly sig.)
  - Pooled Swine farms v. Land application site (strongly sig.)

	Swine #1	Swine #2	Pooled	Land App.
Reference	0.11	0.01	0.003	0.40
Pooled Sw				0.005



# AR Frequencies & MAR of Enterococcus at Swine & Reference Sites

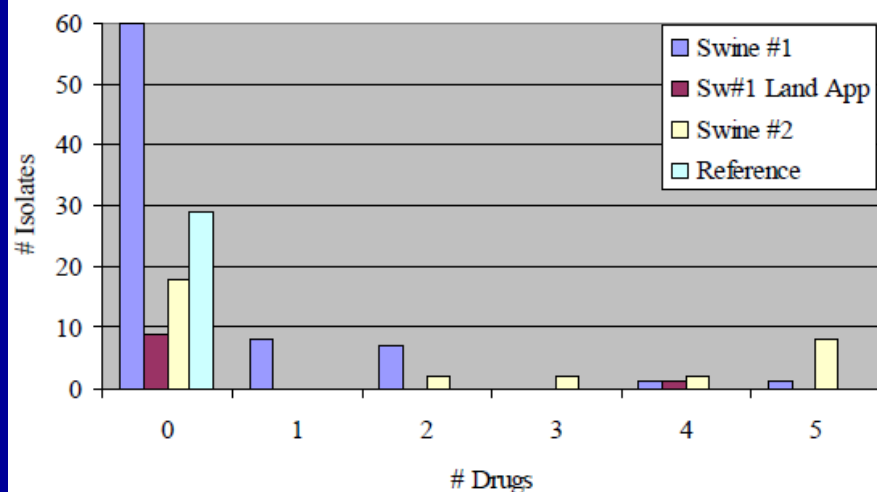
## Wilcoxon Scores Exact Test – Enterococci

Statistically significant differences in AR frequency between:

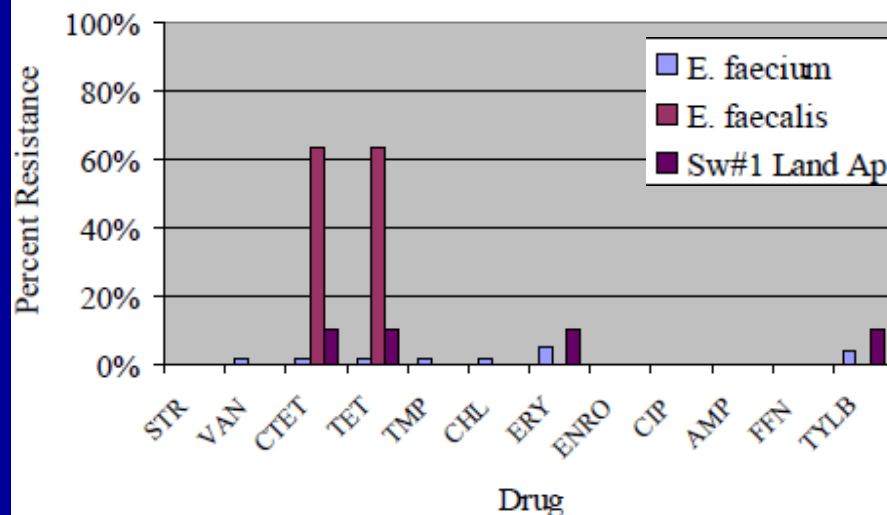
- Swine #1 v. Reference site (significant).
- Swine #2 v. Reference site (significant).
- Pooled Swine farms v. Reference site (significant).
- Pooled Swine farms v. Land application site (strongly sig.).

	Swine #1	Swine #2	Pooled	Land App.
Reference	0.03	0.04	0.02	0.50
Pooled Sw				0.001

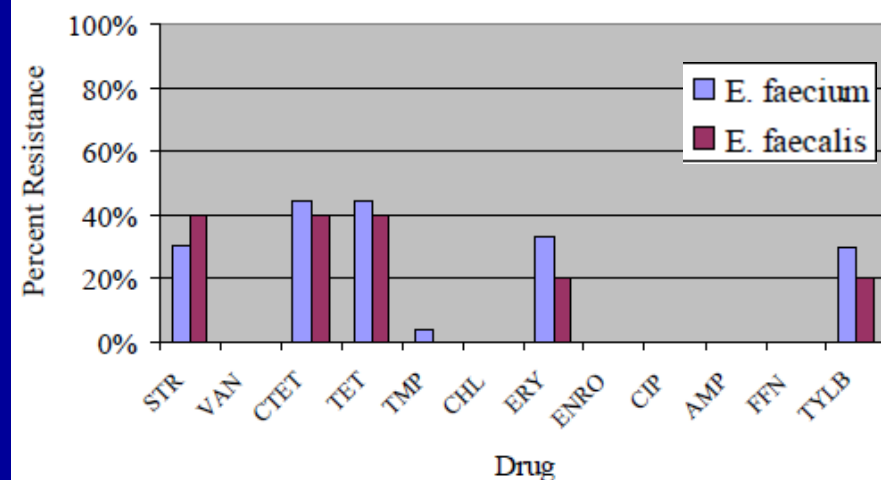
Enterococcus Antimicrobial Resistance - Number of Drugs per Isolate



## Enterococci % Resistance/Drug Farm #1



## Enterococci Percent Resistance per Drug - Swine Farm #2

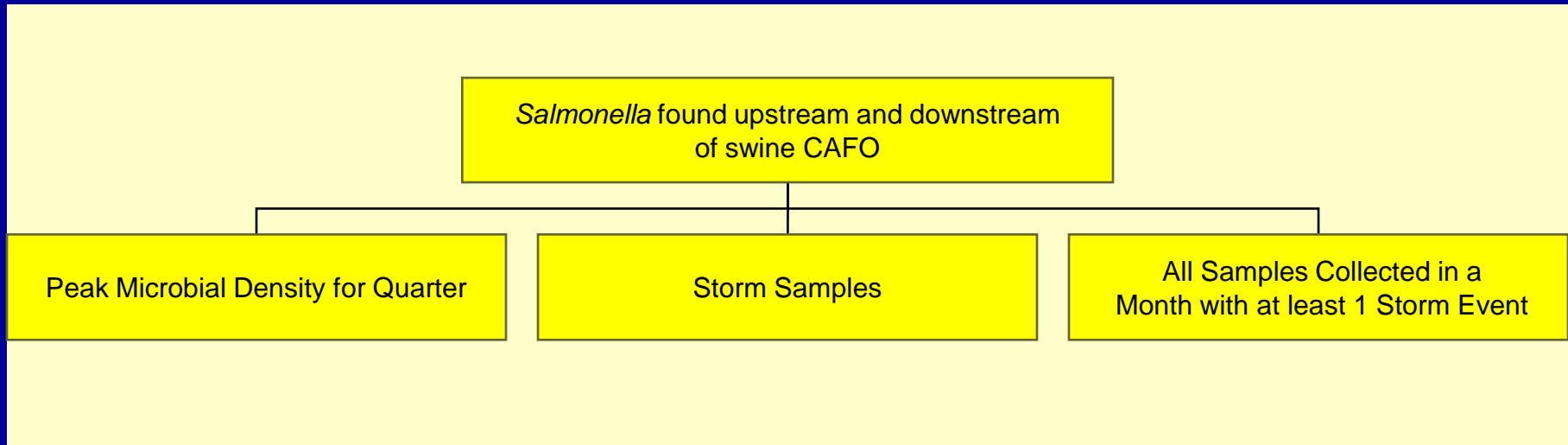


# Documented AR Microbial Impacts of Swine Farms and WWTPs on Surface Waters

## Study Design:

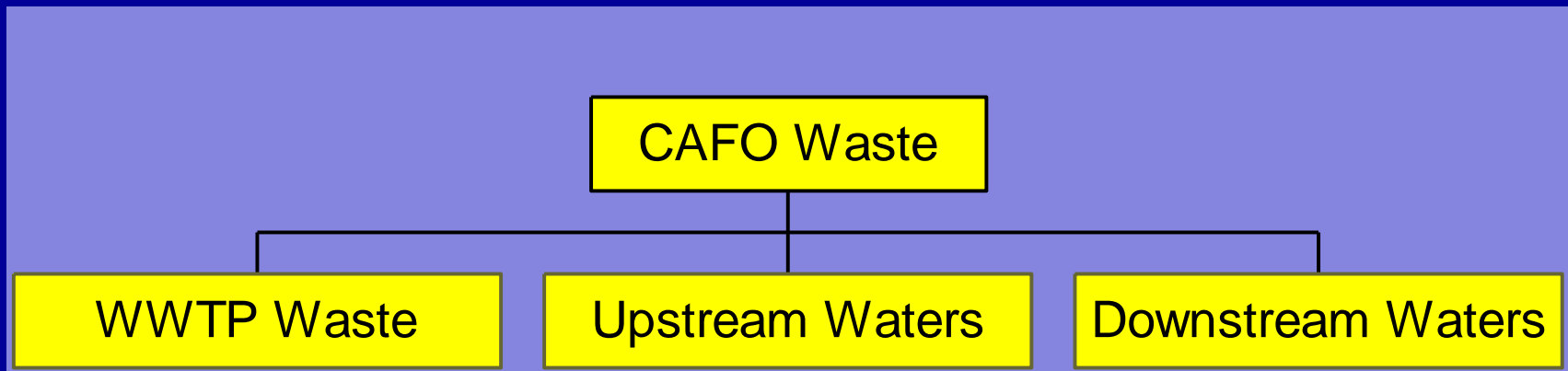
- Two surface water bodies in rural NC
  - 3 “background” sites
  - 2 sites downstream of swine CAFOs (“swine-impacted”)
  - 1 swine CAFO drainage ditch outlet (“swine-impacted”)
  - 1 site downstream from a permitted human waste discharge site (“human-impacted”)
  - 1 site downstream from a number of human septic systems (“human-impacted”)
- Waste sources
  - 2 regional WWTPs
  - Swine barn flush and waste lagoons
  - Resident waterfowl on CAFO 2

# Salmonella Source Tracking at Swine CAFOs



- **Waste-source isolates were matched with selected stream isolates by collection date and serotyped (U. Penn *Salmonella* Reference Center)**
- **108 waste and water source isolates with matching serotypes and antimicrobial resistance patterns were further evaluated (at UPenn *Salmonella* Reference Center):**
  - **Phage typed (Typhimurium)**
  - **Pulsed field gel analyzed (PFGE) using Xba1**

# Comparison of Salmonella from Different Sources



## **Criteria for matching waste isolates to stream isolates**

- Matching serotype**
- Matching antimicrobial resistance pattern**
- Matching phage type or PFGE profile**

# Storm Events Increased Fecal Bacteria Levels in Streams

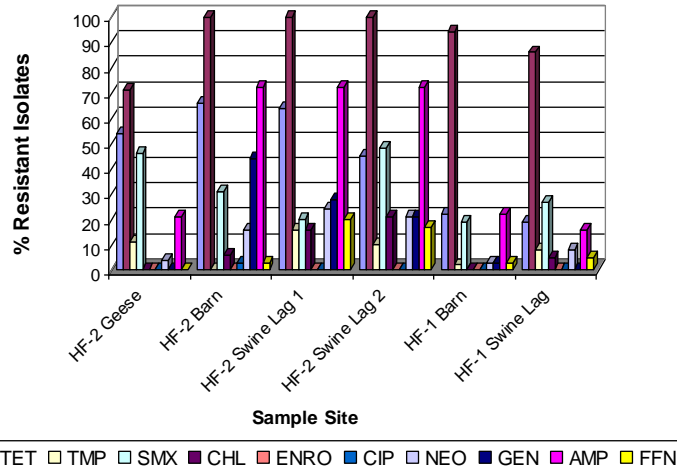
Mean Log Densities (CFUs/100mL)	Base (SD)	Storm (SD)
<i>E. Coli</i>	4.8 (1.2)	7.0 (2.4)
<i>Enterococci</i>	5.6(1.9)	8.9 (2.2)

- Fecal Indicators were isolated from 100% of samples
- *Salmonella* were detected in 79%-100% of samples
  - More frequently detected and higher counts at swine CAFO impacted sites

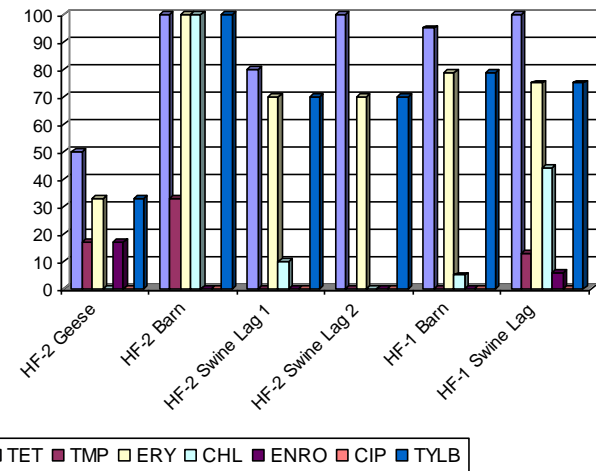
Salmonella Detection and Concentration				
	Frequency of Detection		Mean MPN/1L (SD)	
Collection Conditions	Baseflow	Storm	Base-flow	Storm
Background	37/47 (79%)	6/7 (86%)	10 (18)	35 (29)
Human-impacted	29/33 (88%)		15 (21)	
Swine-impacted	48/52 (92%)	7/7 (100%)	16 (26)	55 (28)

# AR *E. coli* and Enterococci are in Swine Wastes and Adjacent Streams too

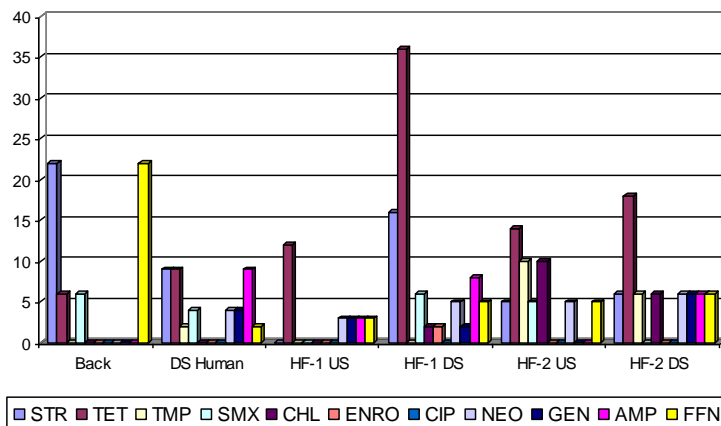
Antimicrobial Resistance Among *E. coli* in Wastes



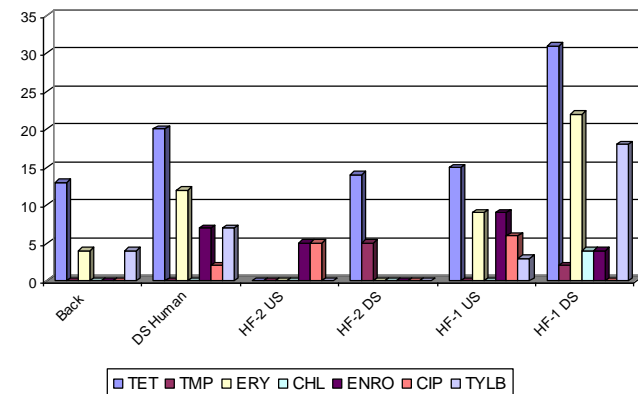
Antimicrobial Resistance among Enterococci from wastes



Antimicrobial Resistance among *E. coli* in Streams



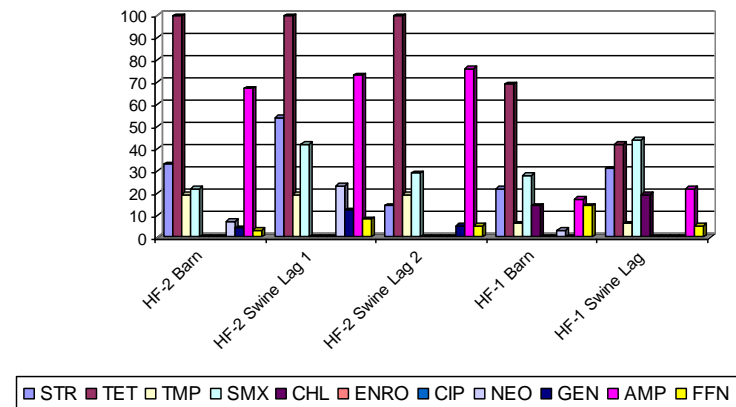
Antimicrobial Resistance among Enterococci in Streams



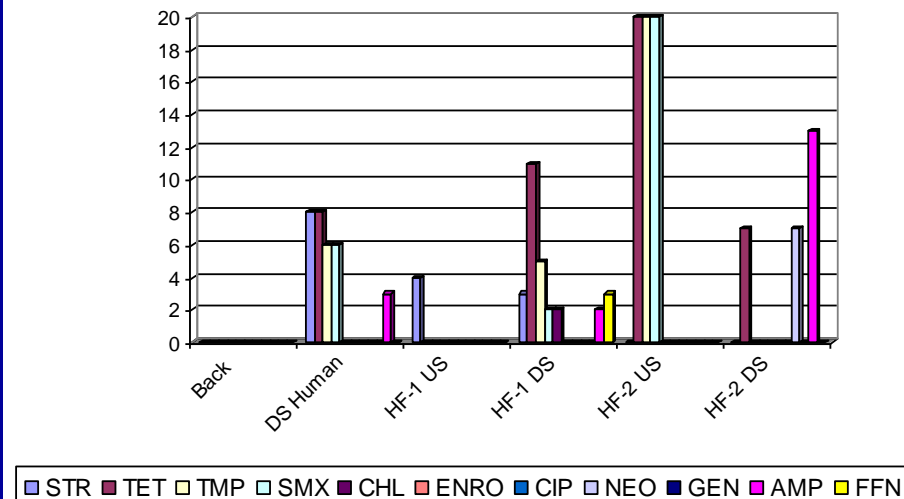
# AR *Salmonella* are in Wastes and Streams

- Downstream serotypes most commonly matched upstream serotypes
  - Stream 1: 51%
  - Stream 2: 25%
- Percentage of stream serotypes matching CAFO 1 waste serotypes:
  - Upstream: 24%
  - Downstream: 42% ( $p=0.066$ )
- No downstream serotypes matched region 2 CAFO serotypes

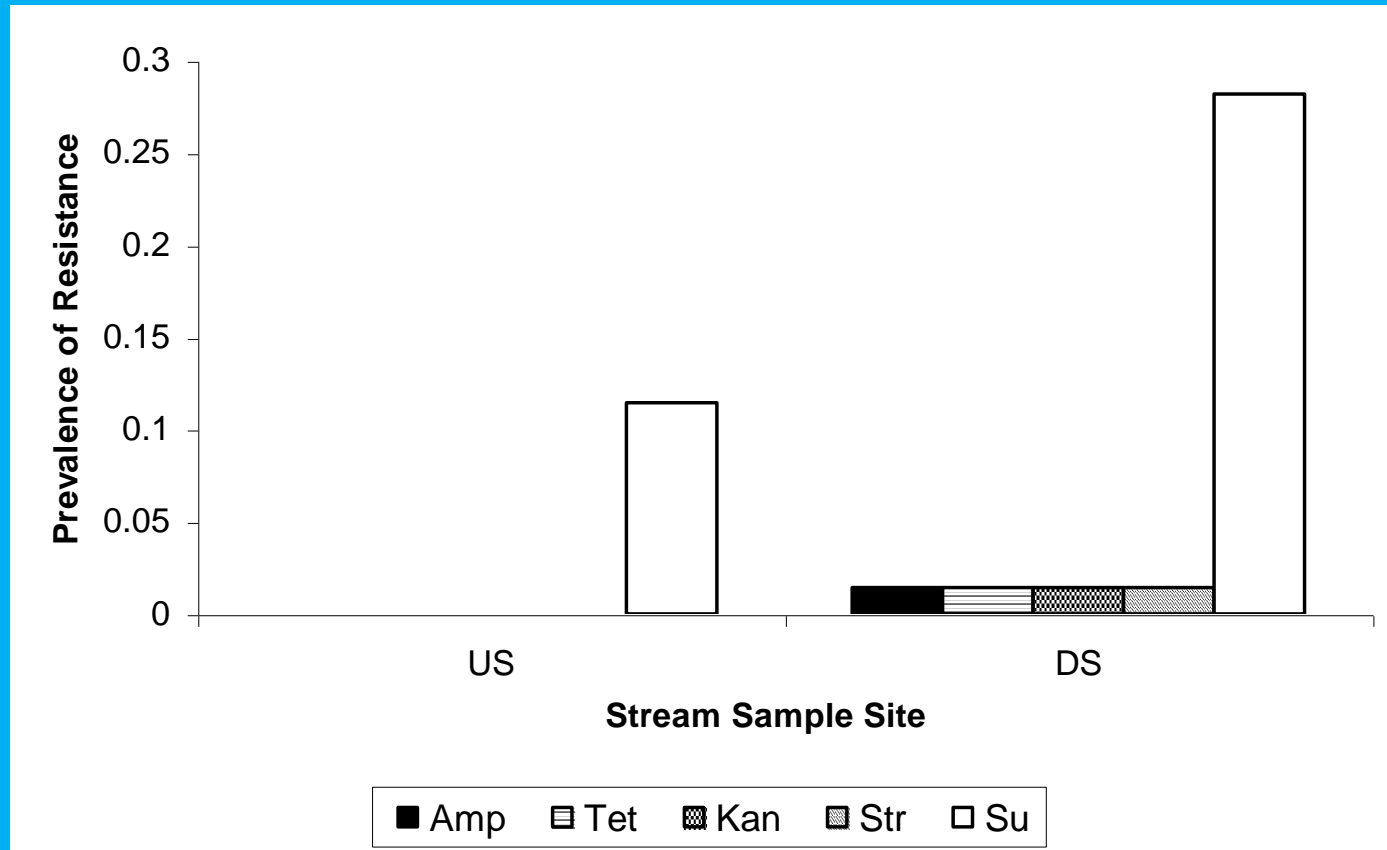
Antimicrobial Resistance among *Salmonella* in wastes



Antimicrobial Resistance among *Salmonella* in streams



# AR among Matched Salmonella Serotypes in Up- and Down-stream Water Samples from Swine CAFOs



# Surface Water Impacts from WWTPs and Swine Farms

- AR bacteria isolates appear to be transported from waste system to surface waters
- Surface waters impacted by swine CAFOs were associated with greater frequency of antimicrobial residue detection  
(antibiotic residue data not shown)
- Bacteria isolated from surface waters impacted by swine CAFOs exhibited a higher prevalence of antimicrobial resistance
- Does exposure to contaminated surface water represent an additional human exposure pathway to antimicrobially-resistant pathogens?

# Summary/Recommendations

- Animal and human waste management activities have AR microbial impacts on surface and ground waters & other environmental media
- Surface and ground waters are at risk of microbial contamination, especially during and after rainfall events
- Management practices for animal wastes need further improvement to:
  - reduce AR microbe levels and pathogen loads to waters used for various beneficial purposes
  - reduce airborne emissions of AR pathogens

# Recommendations and Future Directions

- Better EPA guidance and regulations may be advisable to compliment the guidance and regulations for drinking and recreational water quality criteria to reduce human health risks
- Better NPDES permits to compliment Best Practicable Control Technologies, Best Available Technologies, Best Management Practices,
- CAFO/AFO effluent and emission guidelines
- Better determination of Total Maximum Daily Loads for AR microbes/pathogens
- *We need to directly address AR pathogens*

# A Way Forward

Studies to More Conclusively Determine  
if there are Human Health Risks from AR  
Pathogens in Animal Agriculture Wastes

*A human/animal health risk-based approach is needed*

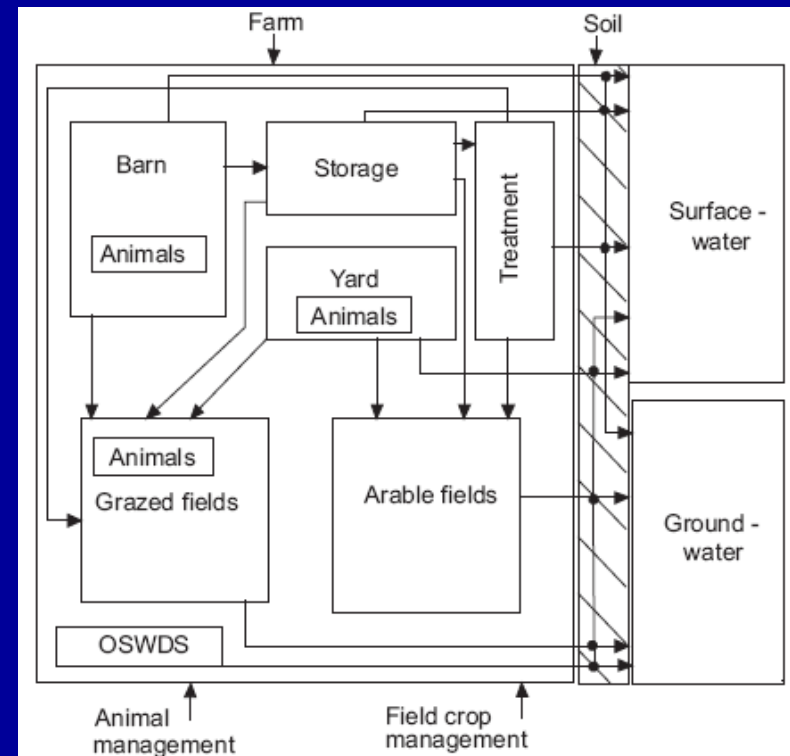
- **Human epidemiological studies**
  - Ag. worker
  - Ag. neighbor
  - Drinking water exposure/health effects
  - Recreational water exposure/health effects

# Using a Risk-based Approach for Pathogen Exposure Assessment

A recent example:

- A farm-based framework for a pathogen risk index for environmental waters
- A basis of exposure assessment of pathogens of animal farm origin

**Gross & Richards (2008)** Development of a risk-based index for source water protection planning, which supports the reduction of pathogens from agricultural activity entering water resources. *J Environ Manage.* 87(4): 623-32

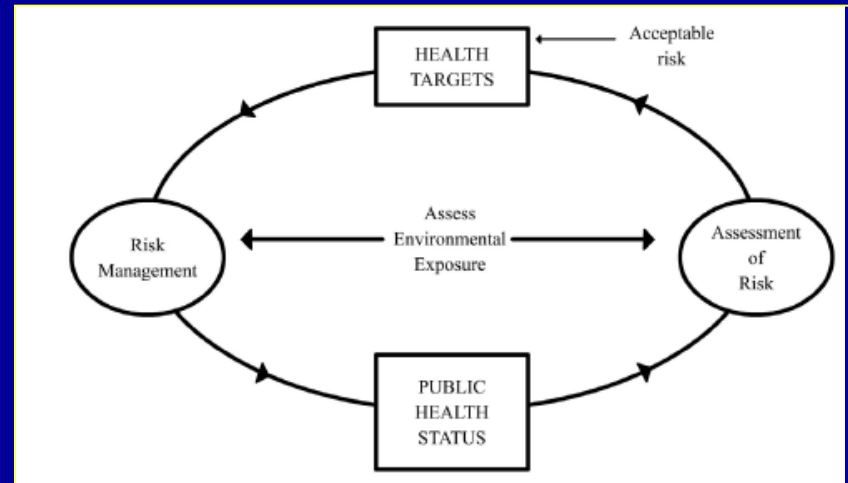


Schematic of a pathogen risk index

Arrows below the framework indicate farmer decisions. Arrows within the framework indicate potential pathways for pathogen movement.

## Assessing and Managing AR Pathogen Risks from Animal Production Systems Requires Innovative and Effective QMRA Approaches

- Use health risk-based approaches to pathogen management in food animal production systems
- QMRA
  - Hazard Identification
  - Exposure assessment
  - Effects assessment
  - Risk characterization
- Employ HACCP systems
  - “Water Safety Plans”
  - Rational management based on pathogen risk levels and their control



# Acknowledgements

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- Shehee, Mina
- Simmons, OD III
- Williams, Mike

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